SLOVENSKI STANDARD
SIST EN ISO 16140-4:2020/oprA1:2023

01-oktober-2023

Mikrobiologija v prehranski verigi - Validacija metode - 4. del: Protokol za validacijo metode v posameznem laboratoriju (hišne metode)- Dopolnilo A1: Validacija testnega vzorca večje velikosti za kvalitativne metode (ISO 16140 4:2020/DAM 1:2023)

Microbiology of the food chain - Method validation - Part 4: Protocol for method validation in a single laboratory - Amendment 1: Validation of a larger test portion size for qualitative methods (ISO 16140 4:2020/DAM 1:2023)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 4: Arbeitsvorschrift für Einzel-Labor-Verfahrensvalidierung - ÄNDERUNG 1: Validierung größerer Prüfmengen für qualitative Verfahren (ISO 16140 4:2020/DAM 1:2023)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 4: Protocole pour la validation de méthodes dans un seul laboratoire - Amendement 1: Validation d'une taille de prise d'essai plus grande pour des méthodes qualitatives (ISO 16140 4:2020/DAM 1:2023)

Ta slovenski standard je istoveten z: **EN ISO 16140-4:2020/prA1**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST EN ISO 16140-4:2020/oprA1:2023 **en,fr,de**

DRAFT AMENDMENT
ISO 16140-4:2020/DAM 1

ISO/TC **34**/SC **9** Secretariat: **AFNOR**

Voting begins on: **2023-07-19**

Voting terminates on: **2023-10-11**

Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

AMENDMENT 1: Validation of a larger test portion size for qualitative methods

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO 16140-4:2020/DAM 1:2023(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 16140 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

AMENDMENT 1: Validation of a larger test portion size for qualitative methods

Clause 3

Add the following definition:

3.13
larger test portion size
measured (volume or mass) representative sample taken from the laboratory sample or test sample for use in the preparation of the initial suspension that is larger than the test portion that has been described in the original method and/or validation document

4.1

Add the following text after the second paragraph:

The protocol which shall be used to validate a larger test portion size for qualitative methods in a single laboratory is described in Annex H.

Annex H

Add the following annex after Annex G, before the Bibliography.

Annex H
(normative)

Validation of a larger test portion size for qualitative methods

H.1 General

This annex describes a protocol for the validation for qualitative methods when using a larger test portion size. This protocol is intended to demonstrate the effect of analysing a test portion larger than the test portion initially used to validate the method. Validation of the larger test portion size applies only to the laboratory conducting the study and only to the specific (food) category used.

Reference methods which were validated using a larger test portion size in accordance with ISO 17468 and alternative (proprietary) methods which were validated using a larger test portion size in accordance with ISO 16140-2 only need to be verified by a laboratory following ISO 16140-3.

The validated larger test portion size can be used in other laboratories once this has been validated in an interlaboratory study in accordance with ISO 16140-2 or ISO 16140-5. See the flow diagram in Figure 1. Once validated in an interlaboratory study, any laboratory can implement the larger test portion size after verification following ISO 16140-3. If not validated in an interlaboratory study, validation in accordance with the protocol in this annex is necessary for each laboratory wishing to use a larger test portion size.

Requirements with regards to pooling are described in ISO 6887-1:2017.

When testing a larger single test portion, pooled test portion or pooled (pre-)enrichment test portion, the dilution ratio (sample/diluent) used in the validated method shall remain the same. This ratio may be increased to overcome the inhibitory effects coming from the sample (examples are described in ISO 6887-4:2017, 9.1.4.4).

A reduction in the dilution ratio (sample/diluent) requires a validation (details are given in Figure 1).

Items can either be composited, pooled as a test portion or as (pre-)enriched test portion but not as two or more combinations [i.e., pooling of (pre-)enriched as well as test portion is not allowed]. When for example a laboratory uses both pooled test portions and pooled (pre-)enriched test portions, two separate validation studies shall be conducted in accordance with this protocol.

Once the larger test portion size has been validated, all test portions smaller than the largest validated test portion can be used for routine testing for this particular (food) category at the same sample/diluent ratio. For example, a method that has been validated for 375 g test portions can be used for 25 g, 100 g, etc., up to 375 g test portions.

The protocol is intended to demonstrate that a larger test portion size provides a similar or lower level of detection (LOD_{50}) compared to the LOD_{50} of the (validated) test portion size as described in the method. The relative level of detection (RLOD) approach described in 6.1.1.3 shall be used. For calculation of the data, the larger test portion size corresponds to the alternative method and the original test portion size corresponds to the reference method.

H.2 Selection of item, number of samples, and replicates tested

The (food) item selected for the validation of a larger test portion size shall be a relevant (food) item which is routinely analysed in the laboratory. When various (food) items belonging to the same (food) category, are of interest for testing with a larger (but the same) test portion size, the most challenging (food) item will be selected for the validation study. The rationale for the selection of the

challenging (food) item shall be documented. Each food category shall be validated by using at least one (challenging) food item.

NOTE 1 Guidance for choosing a challenging (food) item is given in ISO 16140-3:2021, Annex B.

The samples shall be artificially inoculated. Procedures for the preparation of artificially inoculated samples are specified in ISO 16140-2:2016, Annex C.

A minimum of three levels per (food) item shall be prepared consisting of at least a negative control level, a low level, and a higher level. Ideally, the low level shall be the theoretical detection level (i.e. 0,7 cfu per test portion) and the higher level just above the theoretical detection level (e.g. 1 cfu to 1,5 cfu per test portion).

At least, 1 replicate shall be prepared for the negative control level (L0), 20 replicates for the low level (L1), and 5 replicates for the higher level (L2). An estimate for the level of contamination (except for the negative control) shall be made.

The replicates shall be analysed with both test portion sizes [specified (validated) test portion size in accordance with the reference method and the alternative larger test portion size].

All negative control samples shall not produce positive results. When positive results are obtained, repeat the experiment for all levels.

The low level should have fractional recovery using the validated test portion size (fractional recovery at the low level should be between 25 % and 75 % of the number of samples tested).

NOTE 2 To give better assurance that fractional recovery will be obtained, additional levels of contamination can be prepared and tested.

H.3 Calculation and interpretation of the RLOD

The RLOD is defined as the ratio of the LODs of the larger test portion size (larger) and the original (org) test portion size:

$$RLOD = \frac{LOD_{larger}}{LOD_{org}}$$

where

LOD_{larger} is the LOD obtained with the larger test portion size;

LOD_{org} is the LOD obtained with the specified (validated) test portion size in accordance with the method.

The RLOD shall be estimated by fitting a complementary-log-log (CLL) model to the combined detected/not detected data as a function of test portion size. The contamination levels are not required for the calculations of the RLOD since they are included in the model; the resultant curves are plotted in a graph of probability of detection versus log dose (contamination level). The statistical model and the calculations are given in ISO 16140-2:2016, Annex D.

Calculations can be performed using the Excel® based program[1)] for ISO 16140-4. The Excel® based program for calculating RLOD values is freely available for download at https://standards.iso.org/iso/16140/-4/ed-1/en/ and then select the 'RLOD_MCS_in_ISO_16140-4_subclause_5-1-1-4_V3_2015-08-15.

1) Excel® is the trade name of a product supplied by Microsoft and is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

xlsm'-file. For calculations using this Excel® based program, the option of "unknown concentration" shall be used.

NOTE In this Excel® based program, data obtained with a reference and alternative method are used. To use it for this protocol, the term "reference method" is replaced by "the specified (validated) test portion size in accordance with the method " and the term "alternative method" is replaced by "larger test portion size" .

An Acceptability Limit (AL) for the RLOD specifies the maximum increase in LOD of the larger test portion size versus the test portion size in accordance with the method to decide if the larger test portion size used is fit for purpose.

The AL for the larger test portion size (unpaired study design) is set at 2,5; meaning that the LOD for the larger test portion size shall not be higher than 2,5 times the LOD of the (validated) test portion size of the method. The AL is not met when the observed value is higher than 2,5. In this case, carry out investigations (e.g. root cause analysis) to provide an explanation for the observed results. Based on the AL and the additional information, decide whether the larger test portion size is fit for purpose for the (food) item or (food) category involved. Reasons for acceptance of the larger test portion size if the AL is not met shall be justified.

Two examples, using artificial data, to calculate RLOD for validation of larger test portion sizes are given below.

EXAMPLE 1 The ISO method for the detection of *Salmonella* (ISO 6579-1) was evaluated for the use of 375 g test portions for cocoa powder (category: chocolate, bakery products and confectionary). The enrichment was done for both test portion sizes in accordance with ISO 6887-4 by using a 1 in 10-dilution in non-fat dried milk supplemented with brilliant green. The following results were obtained.

Level of inoculation	Number of positive test portions of 25 g out of total number tested	Number of positive test portions of 375 g out of total number tested
Negative control level	0/1	0/1
Low level	13/20	12/20
Higher level	5/5	5/5

Using the RLOD Excel® based program the following output was obtained. The value for the sample size is by default 25 g and this value has no impact on the calculated RLOD value.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Date:	d/m/yy	Name:	<description>										
2	Sample size:	25	# matrices:	3	# samples:	9	Unknown concentrations			Compute RLOD	Clear Results	Clear Input		
3														
4														
5	Name:	<name>					Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
6	Level	n_1	n_2	y_1	y_2		<name>	1,146	0,498	2,636	0,136	0,417	0,327	0,744
7	low	20	20	13	12		<name>							
8	higher	5	5	5	5		<name>							
9							Combined	1,146	0,498	2,636	0,136	0,417	0,327	0,744
10														
11														
12														
13														
14														
15														
16														
17	Name:	<name>												
18	Level	n_1	n_2	y_1	y_2									
19														
20														
21														
22														

As the calculated RLOD of 1,146 is less than 2,5 (AL limit for unpaired study design), the use of 375 g test portions was successfully validated for the detection of *Salmonella* in accordance with ISO 6579-1 for cocoa powder and thus valid for the category 'Chocolate, bakery products and confectionary'.

EXAMPLE 2 An alternative ISO 16140-2 validated method for the detection of *Cronobacter* spp. was evaluated for the use of 300 g test portions for powder infant formula containing probiotics (category: Infant formula and infant cereals). The method was validated for a broad range of foods but the category 'Infant formula and infant cereals' was not included in the validation study. The following results were obtained.